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An Ayurveda Biology approach for understanding the multi - modulatory effects of Ayurveda formulations for managing diabetes and obesity

Synopsis

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Background

The world is seeing an unprecedented rise in metabolic health issues making the global population vulnerable. To confront the global challenge of rising non-communicable diseases (NCDs), an integrative healthcare system incorporating the principles and practices of both modern and traditional systems seems to be a plausible strategy. Among the NCDs, there is a rapid rise in the incidence of diabetes and obesity across the world. Among the difference types of diabetes, type 2 diabetes mellitus (T2DM) is the most common and has shown a very profound association with life-style, diet and obesity. Despite rapid strides in healthcare research and innovations, both T2DM and obesity remain a huge challenge in terms of treatment and management strategies (Bansode and Jungari, 2019). Due to the predominance, biological complexity and higher co-morbidity association, T2DM is attracting more research attention to understand the disease biology and to come up with novel and affordable management solutions. The current modern medicinal perspective is reductionist, where the treatment approaches largely break down the individual's biology into cellular and organ systems and the dynamic nature of systemic interactions and biochemical cross-talks are not given adequate importance (Fang and Casadevall, 2011). The fundamental tenets of health stems from dynamic interaction of biological systems (biomolecules, cells and organs) within the individual (*milieu intérieur* concept developed by the French physiologist Claude Bernard) as well as between the individual and the environment including diet, lifestyle, social interactions and so on and so forth; and these factors are not mutually exclusive (Krahn et al., 2021). The holistic understanding of health and disease as well as their management considers every physiological and biochemical event as one, and attempts to see the entire organism more than the collection of its constituent parts. While the modern medicine made rapid advances in the prevention and cure of various diseases, primarily infections, it has become increasingly evident that it lacks the holistic perspective that is much needed for the management of the rising 'epidemics' of lifestyle diseases like diabetes, obesity and various metabolic diseases associated with them (Paczkowska-Abdulsalam and Kretowski, 2021). The side effects of existing drugs and rising cost of medicines are some of the other reasons for increasing attention to integrative medicinal approaches globally, especially for management of complex life style diseases like T2DM and obesity (Padhi et al., 2020). For managing these chronic lifestyle diseases, positing a holistic system focusing on the patient centered disease management and wellness appears to be more advantageous as compared to the contemporary approach which emphasizes a more symptomatic relief treatment.

Etiology and pathophysiology of Diabetes mellitus

Diabetes is one of the most prevalent epidemics globally. It is currently recognized and classified as a group of diseases characterized by signs and symptoms of chronic hyperglycemia which eventually leads to loss of proper insulin response in tissues, referred as insulin resistance. Around 537 million adults are currently diagnosed with diabetes mellitus and this is expected to rise to 643 million by 2030 (Ong et al., 2023). As per the statistics of International Diabetes Federation (IDF), there are over 74 million people having diabetes in India and around 58% go undiagnosed. Various cross sectional studies indicate that prevalence of diabetes in India has risen significantly in the last 10-15 years (Balasopoulou et al., 2017). There is also evidence that Asian Indians progress more rapidly through the prediabetes stage

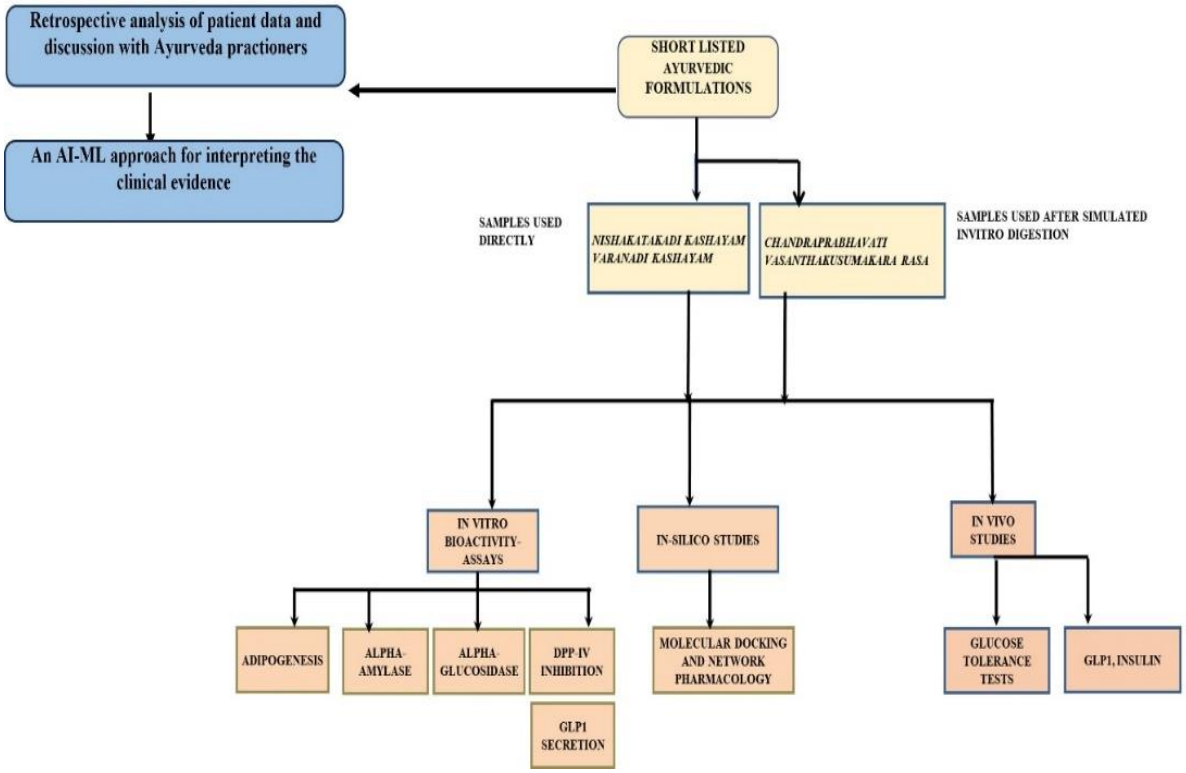
as compared to people of other ethnic groups (Yang et al., 2010). The recent Indian Council of Medical Research–India Diabetes (ICMR-INDIAB) study, a cross-sectional population based survey, reported that the overall weighted prevalence of diabetes is 11.4%, pre-diabetes is 15.3% and generalized and abdominal obesity are 28.6% and 39.5% respectively (Anjana et al., 2023).

Patients with diabetes and obesity are at risk of developing a spectrum of diseases affecting liver, kidney and macrovascular systems. Long term hyperglycemia leads to damage to renal cells as well and it leads to chronic diabetic kidney disease (Huang and Khardori, 2017). Diabetic patients can also have hypertension which can cause cardiovascular complications (Ohishi, 2018). Thus, a multi-factorial etiology makes the treatment and management of diabetes and obesity to be a challenging task.

The current strategy for diabetes and obesity management are mainly targeted therapies along with lifestyle modifications. However, the disease management becomes increasingly difficult due to the chronicity of the diabetic complications (Mosenzon et al., 2021). No single drug can reverse the multiple abnormalities in diabetes which results in life time drug dependency and side effects. The socio-economic burden along with the multifactorial nature of the disease calls for a change in the perspective of treatment and management strategies. Therefore, the need of the hour is a paradigm shift from the targeted to a holistic perspective of disease management with interventions targeting various organ systems involved. In this context, traditional medicinal systems following holistic principles of disease management gains relevance. Ayurveda, one of the most accepted Indian Systems of Medicines (ISMs), which has a person-centered disease management approach that can be a worthwhile option to address the heterogeneity and complexity of the disease. Also, Ayurveda is a rich source of multi component formulations which can target various pathways to alleviate multiple symptoms. Such a multi-targeted therapeutic approach agrees with the emerging findings of various studies of benefits of combinatorial therapy in diabetes and obesity (Davies et al., 2022). Taking cues from Ayurveda, a trans-disciplinary integrative medicinal system can emerge integrating the holistic concepts of Ayurveda and molecular insights of modern biomedicine. One of the emerging disciplines in Ayurveda research is 'Ayurveda Biology' aims to use modern biological techniques to study the Ayurvedic practices and formulations which can lead to generating novel insights into understanding health and disease progression as well as treatment methods. Exploring how Ayurveda formulations act through various approaches like in vitro cell models, in vivo experiments as well as in silico methods can illuminate our understanding into Ayurveda concepts of disease pathophysiology as well as uncover novel targets.

The aim of the present study is to investigate the various modes of actions exerted by four clinically prescribed classical anti diabetic formulations *Varanadi Kashayam* (VA)*, Nisakatakadi Kashayam* (NK), *Vasantakusumakara rasa (*VK) and *Chandraprabha vati (*CV). (Singh, R.H., 2013; Bhatt, 2015.; A shastri, 2003).

The four formulations contain a number of medicinal herbs and was selected on the basis of discussions with Ayurveda practitioners and clinical data from the I-AIM hospital, Bengaluru. The present study hypothesized that the Ayurveda concept of ***Agni*** can be corelated with gastro intestinal glucose disposal (GIGD)- referring to gut mediated glucose regulatory actions.



This figure gives an overview of the various experimental approaches to investigate the multi-modal action of formulations

The formulations were studied for their anti-diabetic and anti-obesity effects using various assays to understand the modulation of therapeutically relevant targets and pathways in diabetes. Assessment of digestive enzyme inhibition, anti-adipogenesis, DPP4 inhibition, GLP1 secretion and expression of relevant molecular markers were performed using *in vitro* and *in vivo* model systems. Also, the thesis employed computational biology tools *in silico* approaches like virtual screening, molecular docking and network analysis based on widely existed databases were done to get a better understanding of the systemic action of these herbal formulations. By focusing on GLP1 and DPP4 using *in vitro*, *in vivo* and *in silico* approaches, the thesis brings to light the gut modulatory actions of these *Ayurveda* formulations, as well as their possible multi-modulatory effect in metabolic disease management.

Chapter 1 gives the literature review on the global prevalence of diabetes and obesity and the socio-economic challenge posed by these diseases. **Under this, section 1.2** establishes the need for the transdisciplinary platform for diabetes and obesity management. It also describes the patho-physiology and progression of diabetes along with the current therapeutic scenario of these diseases. Here, the concept and correlation of ***Agni*** and gut mediated glucose disposal (GIGD) is established.

Chapter 2 gives the methodology and rationale for the experimental approaches that has been adopted. The anti-diabetic and anti-obesity effects of formulations is evaluated by different experiments like digestive enzyme inhibition, DPP4 inhibition, GLP1 secretion and anti-adipogenic assay. Molecular studies were done by quantitative PCR to understand the molecular basis of mode of action of formulations. A computational biology approach was used

for exploring the multi-targeted actions of formulations. Finally, the formulations were also investigated for their anti-diabetic effects in an in vivo diabetic rat model. The detailed protocol for all the experimental setup is described in this section.

Chapter 3 starts discussing the results of experiments carried out with various Ayurveda formulations. It begins with a short description of the methodology used for selection of formulations based on literature review and discussion with Ayurveda practitioners in the Institute of Ayurveda and Integrative Medicine (I-AIM), Yelahanka, Bangalore as well as with experts from the field of Ayurveda. Based on the discussions and literature review, in the current thesis work four 'classical Ayurveda formulations' were selected. (descriptions given in Chapter 3) namely *Nishakatakadi Kashaya (NK), Varanadi Kashaya* (VA)*, Vasantha Kusumakara Rasa* (VK) and *Chandraprabha vati* (CV). Each of these formulations were subjected to various bioassays and the results are discussed in the subsequent sections in Chapter 3. This section also describes our attempt to use artificial neural network based self-organizing map approach for natural grouping of clinical features and project multidimensional data onto a two-dimensional display in a non-linear manner. The details of this study are described in section 3.5.

Section 3.1 and 3.2 of the thesis discusses possible mode of action of two formulations viz. NK and VA through which they exert anti-diabetic effect. Both these formulations found to inhibit the enzyme DPP4, a crucial player in the incretin effect, in a concentration dependent manner. Also, these formulations were found to increase GLP1 secretion in both *in vitro* and *in vivo* model systems. Further, this section also discusses results of computational biology approaches adopted to ascertain the possible multi-targeted network pharmacology action of these formulations and the possible molecular targets they modulate in connection with various comorbidities of diabetes and obesity.

Section 3.3 discusses the mode of action of VK which is found to exert its antidiabetic effect through inhibiting digestive enzyme, alpha-amylase. The section also reports the anti-adipogenic effects of VK and the molecular mechanism underlying the anti-adipogenic effects.

Similarly, **Section 3.4** describes the antidiabetic and anti-adipogenic effects of the fourth formulation CV, where it was observed that CV inhibits digestive enzymes and downregulate the genes involved in adipogenesis.

Section 3.5 of this chapter discusses a pilot study conducted to develop a methodology that can encompass and analyze multiple variables by appropriately clustering them to subgroups that justify the personalization principles which influence the clinical efficacy. This is done using concepts of artificial intelligence and machine learning, where higher dimensional statistical analysis was used to cluster the patients into comparable subgroups with matching features that contribute or influence the diagnostic and clinical outcome parameters, safety and efficacy of interventions as well as the personalization concepts.

Chapter 4 of the thesis collectively discussed the differential modes of action of the formulations and attempts to corelate the Ayurveda concepts of ***Agni*** with results obtained from studies conducted with various formulations. The chapter discusses the key findings of the

present work and how it can be valuable for further understanding of pharmacological actions of formulations. **Chapter 5** concludes the thesis and gives future directions of the thesis.

Collectively, the present work has investigated the mechanisms involved in the anti-diabetic and anti-obesity actions of four well-prescribed, yet least investigated Ayurvedic classical formulations through systematic research using appropriate *in vitro*, *in silico* and *in vivo* pharmacological model systems. Two formulations *Varanadi and Nisakatakadi kashayam* demonstrated incretin modulatory activity *in vitro*, that could be correlated with their ability to modulate the metabolism, or the '*Agni'* as termed in the principles and practices of Ayurveda. *Vasantakusumakara rasa* and *Chandraprabha vati* exhibited inhibition of digestive enzymes and anti-adipogenic action by inhibiting lipid accumulation and downregulation of adipogenic genes in mature adipocytes. The study gives an insight into how Ayurveda formulations can exert their effects differentially acting upon multiple targets, which maybe the very basis of personalized treatment strategy adopted by Ayurveda. Through a combination of *in vitro*, computational and *in vivo* model systems, the thesis provides a suitable transdisciplinary framework for Ayurveda Biology research.

Though there are many studies focused on investigating the mode of action of Ayurveda formulations, a transdisciplinary approach adopted in this work, wherein modern bio medicinal tools have been utilized to understand and corelate Ayurvedic principles, can provide valuable insights for their multi-modulatory actions on glucose and lipid homeostasis. An integrative methodology like this will aid in evidence-based integration of Ayurveda into mainstream health practices. Such studies can set the tone and foster an Ayurvedic research ecosystem.

References

- Anjana, R.M., Unnikrishnan, R., Deepa, M., Pradeepa, R., Tandon, N., Das, A.K., Joshi, S., Bajaj, S., Jabbar, P.K., Das, H.K., Kumar, A., Dhandhania, V.K., Bhansali, A., Rao, P.V., Desai, A., Kalra, S., Gupta, A., Lakshmy, R., Madhu, S.V., Elangovan, N., Chowdhury, S., Venkatesan, U., Subashini, R., Kaur, T., Dhaliwal, R.S., Mohan, V., Mohan, V., Anjana, R.M., Unnikrishnan, R., Pradeepa, R., Deepa, M., Sudha, V., Nirmal, E., Subashini, R., Venkatesan, U., Nath, L.M., Lakshmy, R., Tandon, N., Mahanta, J., Madhu, S.V., Das, A.K., Pandey, A., Dhaliwal, R.S., Kaur, T., Rao, P.V., Rao, M.N., Jampa, L., Kaki, T., Das, H.K., Borah, P.K., Kumar, A., Sharma, S., Bhansali, A., Dash, K., Shrivas, V.K., Krishnan, A., Desai, A., Dias, A., Saboo, B., Padhiyar, J.M., Kalra, S., Kalra, B., Mokta, J.K., Gulepa, R., Dhandhania, V.K., Adhikari, P., Rao, S., Jabbar, P.K., Jayakumari, C., Jain, S.M., Gupta, G., Joshi, S., Yajnik, C.S., Joshi, P.P., Ningombam, S., Singh, T.B., Budnah, R.O., Basaiawmoit, M.R., Rosangluaia, Lalramenga, P.C., Suokhrie, V., Tunyi, S., Tripathy, S.K., Sahu, N.C., Purty, A.J., John, M., Gupta, A., Gupta, B.L., Shrivastava, S.K., Tobgay, K.J., Kaleon, T.T., Reang, T., Das, S.K., Bajaj, S., Mathur, M.K., Modi, S., Kakkar, R., Chowdhury, S., Ghosh, S., 2023. Metabolic non-communicable disease health report of India: the ICMR-INDIAB national cross-sectional study (ICMR-INDIAB-17). Lancet Diabetes Endocrinol. 11, 474–489. https://doi.org/10.1016/s2213-8587(23)00119-5
- Bansode, B., Jungari, D.S., 2019. Economic burden of diabetic patients in India: A review. Diabetes Metab. Syndr. Clin. Res. Rev. 13, 2469–2472. https://doi.org/10.1016/j.dsx.2019.06.020

- Huang, L., Khardori, R., 2017. Pathogenesis of diabetic nephropathy. Manag. Diabet. Nephropathies Clin. Pract. 23–45. https://doi.org/10.1007/978-3-319-08873-0
- Casadevall, A., & Fang, F. C. (2008). Descriptive science. Infection and immunity, 76(9), 3835–3836. https://doi.org/10.1128/IAI.00743-08
- Ohishi, M., 2018. Hypertension with diabetes mellitus: Physiology and pathology review-article. Hypertens. Res. 41, 389–393. https://doi.org/10.1038/s41440-018-0034-4
- GBD 2021 Diabetes Collaborators (2023). Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet (London, England)*, *402*(10397), 203–234. https://doi.org/10.1016/S0140-6736(23)01301-6
- Padhi, S., Nayak, A.K., Behera, A., 2020. Type II diabetes mellitus: a review on recent drug based therapeutics. Biomed. Pharmacother. 131, 110708. https://doi.org/10.1016/j.biopha.2020.110708
- Unnikrishnan, R., Anjana, R.M., Mohan, V., 2016. Diabetes mellitus and its. Nat. Publ. Gr. https://doi.org/10.1038/nrendo.2016.53
- Krahn, G. L., Robinson, A., Murray, A. J., Havercamp, S. M., & Nisonger RRTC on Health and Function (2021). It's time to reconsider how we define health: Perspective from disability and chronic condition. *Disability and health journal*, *14*(4), 101129. https://doi.org/10.1016/j.dhjo.2021.101129
- Paczkowska-Abdulsalam, M., & Kretowski, A. (2021). Obesity, metabolic health and omics: Current status and future directions. *World journal of diabetes*, *12*(4), 420–436. https://doi.org/10.4239/wjd.v12.i4.420
- Sushruta: Sushruta Samhita, Part I with Ayurveda Tattva Sandipika Hindi Commentary, Edi. by Kaviraja Ambikadatta Shastri, Published by Chaukhambha Sanskrit Sansthan, Varanasi, 14th edition, 2003
- Singh, R.H., 2013., 2013. Ashtanga Hridayam.
- Rasendra Sara Sangraha, Sri Gopal Krishna Bhatt,2015 edition, English Translation by DR G Prabhakara rao